

THE EFFECTS OF TEMPERATURE GRADIENTS ON THE PUPAL-ADULT TRANSFORMATION OF SILKWORMS

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There is good evidence that the adult development of the higher holometabolous insects is evoked by one or more factors arising in the anterior end of the larva and pupa (Hadorn, 1941; Scharrer, 1941). In the present investigation further information was sought concerning the possible action of such a differentiation center in the pupal-adult transformation of silkworms (Williams, 1940). By the application of temperature gradients, one end of the pupa could be exposed to a temperature ordinarily high enough to induce development and the other end to a temperature ordinarily low enough to maintain dormancy. The developmental dependency of the two ends and of the various tissues could thus be tested.

There is apparently only one previous record of the use of temperature gradients in studying insect development; namely, the investigation of Giersberg (1929) concerning the pigmentation of Lepidoptera.

MATERIALS AND METHODS

The experiments were performed on two species of native silkworms, *Samia walkeri* (*cynthia*) (F. & F.) and *Telea polyphemus* (Cram.). The diapausing pupae were collected during winter, after having been exposed to low temperature, and were stored at 3° to 5° C. until used. Since escape from diapause is facilitated by prolonged exposure to cold (Uvarov, 1931), it may be presumed that the pupae were in a physiological state to develop when heated. A further advantage to the use of such material was the fact that all individuals at the beginning of the experiments were at the uniformly low degree of adult differentiation characteristic of diapausing pupae.

The apparatus consisted of an incubator from which the glass in the hinged door had been removed and substituted by a corrugated cardboard diaphragm. The pupae, in various orientations, were fitted snugly into holes of suitable size in this partition, the incubator set at a favorable developmental temperature (25° to 30° C.), and the entire

apparatus placed in a cold-room having a temperature of 3° to 5° C. A beaker of water inside the incubator maintained the humidity at a high level.

Most of the animals were oriented with the diaphragm midway of their longitudinal axes. There was thus established a temperature gradient along the body of each individual. Fortunately, no major neutralization of this effect would be expected from the circulation of the blood due to the very rudimentary state of this system in the diapausing pupa.

The animals were exposed to the temperature gradient for a maximum of 139 days. During this time the pupae which showed signs of development were removed and studied. Most of the animals were photographed and fixed in Bouin's solution. A few were exposed to room temperature to test its effect on subsequent development.

EXPERIMENTAL RESULTS

Head and Thorax Heated—Abdomen Chilled

As can be seen from Table I, development took place in all pupae thus oriented, with the exception of two individuals that died. Striking differences, however, were found in the degree of adult differentiation at the two ends. After exposure to the temperature gradient for

TABLE I

Development attained by pupae exposed to longitudinal temperature gradients

	Front heated; abdomen chilled	Abdomen heated; front chilled
No development.....	0	6
Front moulted; abdomen immature.....	21	0
Abdomen moulted; front immature.....	0	5 ¹
Both ends immature		
Front more mature than abdomen....	4	0
Abdomen more mature than front....	0	15
Dead.....	2	2
	27	28

¹ Only the anterior $\frac{1}{4}$ to $\frac{1}{3}$ of pupae chilled.

an average of 58 days most of the animals moulted the pupal cuticle at the anterior end posteriorly to about the second abdominal segment. The rest of the abdomen remained firmly enclosed within the pupal cuticle from which it could be removed only by tearing the body wall. The anterior ends of these animals were wholly adult, except that the

wings were not expanded. All the adult structures of the head and thorax were well differentiated and pigmented, as shown in Figure 4. These pupal-adult animals could walk in normal fashion and move the wings slowly. Four individuals were removed from the diaphragm before the anterior end had completely transformed.

Dissection of all of the animals that had been oriented in this manner showed that some adult development had also occurred in the chilled abdomen. However, the degree of adult differentiation of the abdomen was in all cases less than that of the anterior region. The large pupal midgut was frequently still present, although modifications of the gut in the adult direction had clearly taken place. For example, slight convolutions had appeared in the straight hindgut of the pupa and a terminal rectal expansion had been differentiated. The genitalia had initiated development, but were still immature. The first stages in the formation of the adult abdominal cuticle were apparent, although the abdominal muscles were still attached to the pupal cuticle.

Thus it is clear that development in the adult direction had begun at both ends in animals heated anteriorly and cooled posteriorly. The anterior warm end attained full or extensive development whereas the chilled posterior end remained in an earlier stage of adult differentiation.

Head and Thorax Chilled—Abdomen Heated

Six of the 28 pupae oriented in this fashion underwent no development whatsoever, although their abdomens were exposed to the warm temperature for an average of 97 days. At the end of this time these individuals were still alive, but unchanged externally and internally (Fig. 1).

Twenty pupae initiated development. For most of these animals adult differentiation was still incomplete at both ends after an exposure to the temperature gradient for an average of 74 days. Here again the warm end showed the more extensive adult differentiation, although the contrast between the developmental conditions at the warm and cold ends was not as great as when the gradient was the reverse. The adult cuticle of the abdomen was in most cases well chitinized, while that of the head and thorax less mature. Furthermore, fully developed adult genitalia occurred on animals whose thoracic muscles were incompletely formed. It may be noted also that in every case where the earliest stages of differentiation of the adult had begun in the abdomen, adult development had also been initiated in the head and thorax.

In only five instances did the abdomen become wholly mature and moult during the term of the experiments. These animals had been



PLATE I

treated with a less severe temperature gradient, however, since only one-fourth to one-third of each individual was placed on the cold side of the diaphragm, in contrast to the usual orientation of the diaphragm midway of the longitudinal axis. The pupal cuticle of the abdomen became thin and transparent in these five animals so that it was easily removed, whereas the cuticle of the head and thorax remained firm and could be dissected off only with difficulty. A comparison of the opposite ends showed that the anterior region had attained the adult condition also, except that pigmentation and finer histological maturation were generally absent. As shown in Figure 3, all the adult structures of the head and thorax were present but generally soft and incompletely chitinized. The heated abdomen had completely transformed into the adult condition.

In summary, the results obtained from animals chilled anteriorly and heated posteriorly indicate that the development of the abdomen is retarded or prevented by cooling the head and thorax. In those cases where the abdomen became fully developed and moulted, the head and thorax were in the last stages of adult differentiation.

One Side Chilled—Other Side Heated

Pupae treated in this manner produced animals which were wholly adult, except that development proceeded somewhat more rapidly on the warm side. This was especially clear in regard to the degree of pigmentation: the hot side attained complete pigmentation before this process had scarcely begun on the chilled side. A conclusive test of this orientation was impossible, however, due to the difficulty in establishing a sufficiently steep temperature gradient transversely through the relatively narrow pupa.

PLATE I

FIG. 1. Diapausing pupa of *S. walkeri*.

FIG. 2. Hind wings of an individual (*S. walkeri*) that had been heated posteriorly and chilled anteriorly. The wing tips, which were on the warm side of the diaphragm, have become pigmented, whereas the chilled anterior region remains in an earlier developmental condition.

FIG. 3. Result of chilling the anterior region of *S. walkeri* and heating the posterior region. Both ends have transformed, but the chilled anterior region is less mature than the heated abdomen.

FIG. 4. Result of heating the anterior half of *T. polyphemus* and chilling the posterior half. The heated region has completely transformed and moulted, whereas the chilled end remains firmly attached to the pupal cuticle. The wings and legs also show gradients in development.

Subsequent Effects of Developmental Temperature

Certain of the animals that had moulted the pupal cuticle at one end were removed from the diaphragm and placed at room temperature. In about ten days a second partial moult occurred in which the rest of the pupal cuticle was shed to produce the complete adult.

At such a time those animals which had previously moulted the abdominal cuticle showed curious movements of the abdomen: waves of contraction passed forward along its length in a manner similar to that which occurs during the normal eclosion of the adult and expansion of the wings. The wings in no case were expanded, however.

Thus it was possible to produce the complete adult by two partial moults.

Local Action of Temperature

In most of the pupae the diaphragm passed across the wing flaps with the result that the tip of each wing was exposed to one temperature and the base to the other extreme. Development proceeded normally on the heated end, whereas the chilled end remained in an earlier stage of development. This was especially clear in regard to pigmentation, as shown in Figures 2 and 4. The pigmentation of the heated portion was generally complete and normal in every respect up to a narrow transitional zone of incomplete pigmentation at the former level of the diaphragm.

Other examples of local temperature action were found along the lengths of the legs and the antennae.

DISCUSSION

An analysis of the effects of temperature gradients gives us some information in regard to the initiation of adult formation after diapause. We have noted that all of the animals which began to transform showed more rapid differentiation at the warm than at the cool end; yet in no instance did development occur in the heated region without some indication of development likewise being present in the chilled region. This observation, that dormancy is apparently terminated simultaneously over the entire animal, suggests the existence of some mechanism which evokes the formation of the adult.

The experimental results permit a rough localization of such a stimulating factor. Without exception, development took place in the surviving pupae whose heads and thoraces were heated. From this we may infer that dormancy is not maintained by cooling the abdomen. In

contrast, when the anterior end was chilled, 21 per cent of the individuals underwent no development whatsoever after 97 days: overall dormancy thus persisted in these animals when the head and thorax were inhibited by the low temperature. These results may be most simply explained in terms of the existence in the anterior end of the pupa of a developmental center which must become active for adult transformation to ensue.

Further evidence in favor of the presence of such a center is given by the excessive time necessary for the development of the remaining 79 per cent of the animals chilled anteriorly. These individuals usually showed only slight differentiation after an average of 74 days. On the contrary, the head and thorax when heated became fully transformed in the majority of animals after 58 days. The fact that 21 per cent of the specimens chilled anteriorly failed to begin development after prolonged exposure suggests that the longer time necessary for adult differentiation of all animals thus treated was primarily due to a delay in the initiation of development rather than to a retardation once it had begun. If the developmental center is in the anterior end of the pupa, such a delay would be expected.

This evidence in support of the existence of an adult-differentiation center is in agreement with the results obtained by a number of previous investigators using other techniques, as recently reviewed by Bodenstein (1939). It may be noted that the action of a differentiation center in the lepidopterous pupa has been found for both diapausing (Bytinski-Salz, 1933) and non-diapausing (Hachlow, 1931; Piepho, 1938a and b; Bodenstein, 1938, 1939) types of development. The results of the present investigation indicate that the differentiation center does not exert its effect in diapausing silkworms until after a prolonged period during which development is suspended. Such an extensive delay is not found for non-diapausing species and individuals. In view of this fundamental difference it is possible that diapausing and non-diapausing developments differ primarily in terms of the time at which the center attains an activity sufficient to evoke adult development.

The local effects of temperature gradients are also of interest in regard to certain of the differentiation processes which are generally considered to be under hormonal "control." For instance, there is considerable evidence from previous studies that moulting results from the action of a hormone arising in the head of the animal (Wigglesworth, 1939; Scharrer, 1941). It is therefore noteworthy that the majority of the animals heated anteriorly moulted the pupal cuticle of the head and thorax without shedding that of the posterior abdominal region.

This local action of hormonal factors is further illustrated by those wings which showed a gradient in pigmentation. The pupal wing-flaps correspond to flattened sacs into which the blood enters from the thorax by anterior openings at their bases. Hence the chemical environment of the wing tissues, in terms of the blood, must be uniform. Now it is well known that the pigmentation of the wings involves the reaction between the scales and a chromogen circulating in the blood (Braun, 1939). Nevertheless, under the influence of temperature gradients the pigmentation generally became complete in the heated region before the process had scarcely begun in the chilled region (Figs. 2 and 4). This phenomenon was also observed by Giersberg (1929) after various regions of the wing-flaps were chilled by cold water passed through tubing.

This evidence in regard to the local action of the chemical environment shows that, at least in these cases, hormones are the servants rather than the masters of development: the ultimate result is determined by the ability of the tissues to react.

SUMMARY

1. Diapausing pupae of the silkworms, *Samia walkeri* (*cynthia*) (F. & F.) and *Telea polyphemus* (Cram.), were placed in a diaphragm and one end exposed to a temperature of 25° to 30° C. and the other end to a temperature of 3° to 5° C. There was thus established a longitudinal temperature gradient in each individual.

2. Development took place in the majority of animals thus treated. For each of these pupae evidence was found that the termination of diapause occurred simultaneously throughout the animal. This observation suggests the existence of a developmental center which evokes the overall initiation of adult differentiation.

3. An analysis of the results obtained when the head and thorax were heated with those obtained when this region was chilled permits a rough localization of such a developmental center in the anterior end of the pupa. Since a similar relationship has been noted for non-diapausing pupae by previous investigators, it is possible that diapausing and non-diapausing developments differ primarily in regard to the time at which the developmental center attains an activity sufficient to stimulate adult differentiation.

4. After the pupal-adult transformation has once been initiated, the tissues are capable of a high degree of developmental autonomy. Animals were produced which were wholly mature at the heated end while the cold end remained in an earlier stage of adult formation. This result was also observed within single organs. In such cases the develop-

mental velocity of each region was related to the local metabolic conditions as affected by the temperature gradients.

5. Of particular interest were the effects of temperature gradients upon moulting and pigmentation, processes that are generally recognized as involving the action of chemicals of a hormonal nature. The contrasting results observed in the hot and cold regions, notwithstanding their uniform blood supply, emphasizes the importance of tissue competency in carrying out hormonal reactions.

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